Q1 **ARTICLE TYPE**

Treatment with Ethanolic Extract of *Senna Auriculata*, *P. emblica.* L., *Syzygium cumini* (L.) Skeels used by Rural Areas of Rajasthan State, India on Diabetic Complications like Nephropathy and Cardiomyopathy in Rats

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ABSTRACT

Purpose of the research: India is "diabetes capital of the world." Diabetes Atlas-2006: Rise to 69.9 million by 2025 unless urgent preventive steps are taken, considered significant causes of morbidity and mortality.

Methods: Rats treated alloxan (150 mg/kg) i.p. results in diabetic rats given ethanol extract of *Senna auriculata* (*S. auriculata*) leaf, *Syzygium cumini* (*S. cumini*) (L.) Skeels seeds, and *S. cumini* (L.) Skeels seeds (150 mg/kg) p.o., for 42 days. Biochemical parameters of diabetic neuropathy, nephropathy, cardiomyopathy, and histopathology of sciatic nerve, kidney, and heart were done at the end.

Results: In diabetic group found blood glucose level (BGL) (84.42 ± 6.384 to 369.36 ± 7.784 mg/dL); blood protein (7.48 ± 0.051 to 25.18 ± 0.046 mg/dL); urine protein (0.692 ± 0.061 to 2.68 ± 0.056 mg/dL); blood albumin (1.94 ± 0.043 to 0.248 ± 0.007 mg/dL); urine albumin (0.082 ± 0.009 to 2.68 ± 0.056 mg/dL); blood myoglobin (0.042 ± 0.00274 to 0.056 ± 0.00207 ng/dL); urine myoglobin (0.0048 ± 0.00142 to 0.0098 ± 0.00107 mg/dL); blood urea nitrogen (BUN) (23.04 ± 1.093 to 124.81 ± 1.238 mg/dL); serum creatinine (84.06 ± 6.723 to 218.56 ± 7.586 µmol/dL). Etholic extract of *S. auriculata* leaf, *P. emblica* (*P. emblica*) L. fruits, and *S. cumini* (L.) Skeels seeds and combination treated groups found BGL 124.42 ± 7.042, 112.07 ± 6.942, 126.25 ± 7.051 & 98.83 ± 6.932 mg/dL; blood protein 7.98 ± 0.039, 8.02 ± 0.053, 8.06 ± 0.039, and 7.48 ± 0.045 mg/dL; urine protein 1.22 ± 0.058, 0.94 ± 0.049, 0.96 ± 0.056, and 0.82 ± 0.062 mg/dL; blood albumin 1.64 ± 0.033, 1.82 ± 0.036, 1.87 ± 0.044, and 1.96 ± 0.039 mg/dL; urine albumin 0.122 ± 0.008, 0.098 ± 0.007, 0.132 ± 0.009, and 0.108 ± 0.011 mg/dL; blood myoglobin 0.045 ± 0.00189, 0.036 ± 0.00177, 0.041 ± 0.00223, and 0.043 ± 0.00175 ng/dL; urine myoglobin 0.0042 ± 0.00129, 0.0052 ± 0.00119, 0.0064 ± 0.00126, and 0.0036 ± 0.00125 mg/dL; BUN 35.81 ± 1.186, 36.06 ± 1.123, 34.53 ± 1.177, and 29.03 ± 1.229 mg/dL; serum creatinine 98.42 ± 5.526, 99.73 ± 6.064, 101.97 ± 6.052, and 94.83 ± 6.678 µmol/dL.

Conclusions: Ethanol extract of three plants (150 mg/kg) and combination normalizes biochemical parameters and morphological changes myocardium and kidney. The combination was found to be more effective in these diabetic complications.

Keywords: Cardiomyopathy, Diabetic nephropathy, *Senna auriculata* leaf, *Syzygium cumini* (L.) Skeels seeds, *Phyllanthus emblica* L. fruits.

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INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin or because cells do not respond to the insulin that is produced.[1]

India leads the world with the largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world." According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. Even though the prevalence of microvascular complications of diabetes like retinopathy and nephropathy are comparatively lower in Indians, the prevalence of premature coronary artery disease is much higher in Indians compared to other ethnic groups. The most disturbing trend is the shift in the age of onset of diabetes to a younger age in recent years. This could have long-lasting adverse effects on the nation's health and economy. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over

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the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle-aged people.[2]

Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the 217 more common forms of diabetes, namely type 2 diabetes, which accounts for more than 90 percent of all diabetes cases. Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2002. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India, and this is further set to rise to 69.9 million by the year 2025. The prevalence was 2.1 percent in urban population and 1.5 percent in the rural population, while in those above 40 years of age, the prevalence was 5 percent in urban and 2.8 percent in rural areas. The prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The association between diabetes and adverse cardiovascular outcomes, such as heart failure (HF) with or without preserved systolic ventricular function, is well known. Overall, 36 to 47% of all patients with clinical HF and 32 to 33% of those with HF and a normal ejection fraction (EF) have diabetes. Although they are frequently associated, the cardiomyopathy of diabetes seems to develop independently of coronary artery disease, valvular heart disease, or hypertension. Diabetic cardiomyopathy progresses from impaired ventricular relaxation to diastolic dysfunction, with high left ventricular filling pressures, and finally to overt HF.[2-4]

- Diabetic cardiomyopathy (DCM) is a clinical condition diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension.[5]
- DCM may be characterized functionally by ventricular dilation, myocyte hypertrophy, prominent interstitial fibrosis, and decreased or preserved systolic function in the presence of diastolic dysfunction.[5]

Symptoms[6-8]

Signs and symptoms of diabetic cardiomyopathy may be absent. Congestive heart failure symptoms include shortness of breath, swelling in the abdomen, or lower extremities and intolerance to exercise. The fluid that accumulates in the chest because of the decreased ability of the heart to pump effectively may lead to chest congestion and cough, in addition to feelings of pressure in the chest.

- Decreased diastolic compliance
- Systolic dysfunction
- Left ventricular hypertrophy
- Higher LV wall thickness and mass in diabetic hearts
- Ventricular hypertrophy and dysfunction

As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes.[9,10]

Therapeutics agents like insulin, sulfonylureas, biguanides, and thiazolidinedione derivatives and α glucosidase inhibitors are preferred.[11] to reduce the hyperglycemic condition. The drugs that are preferred for treatment, such as sulfonylureas which stimulates pancreatic islets to secrete insulin. Biguanides are responsible for the reduction of hepatic glucose output. Thiazolidinedione derivatives exert their peripheral action by lowering insulin resistance in peripheral tissue. αglucosidase inhibitors augment glucose utilization and responsible for the suppression of glucose production.[12] Apart from the therapeutic option for diabetes like oral hypoglycemic and insulin have some adverse effects.[13] Hence, the current therapy is focused on herbal medicines,[14] and they are used for current therapy due to presumed effectiveness, relatively low cost, presumed fewer side effects, and low toxicity.[15] The medicinal plants might provide a useful source of new oral hypoglycemic compounds, and this may lead to the development of pharmaceutical entities, and this may act as a dietary ~ 32 ~ The Pharma Innovation Journal adjunct to existing therapies.[16] Worldwide there are more than 1,200 plant species, some of the medicinal plants that are used to control blood glucose levels such as *Azadirachta indica*, *Catharanthus roseus*, *Allium sativum*, *Memordica judaica*, Aloe vera, and *Trigonella foenum-graecum*. Due to the presence of active principles in medicinal plants, they have been reported to possess some characteristic properties like pancreatic β cell-regenerating, insulin-releasing, and fighting the problem of insulin resistance. India is well known for its great heritage of herbal medicinal knowledge. A large number of tribals and ethnic people living in remote forest areas depend on plants to a great extent for foods, medicine, pharmaceuticals, and agrochemicals. From the decades' studies on ethnobotany have gained importance. Diabetes is an important chronic disorder afflicting many from various walks of life around the world. Though they are various allopathic drugs used to treat the worse effects of diabetes, herbal formulations are preferred to minimize the risk of side effects and due to low cost.[17-19]

According to WHO's estimation, 80% of the world's population uses herbal medicine. Nowadays, traditional medicine with good clinical practice is showing a lively future in treating diabetes and its complications. From the decades' vigorous research on ethnobotany shows that plant and its derivatives are useful in the treatment of diabetes mellitus. Though there are numerous approaches to treat diabetes, traditional medicine is preferred due to its lesser side effects and low cost. In Indian systems of herbal medicine, most traditional practitioners formulate and give out their own recipes. India is the largest producer of medicinal plants, and approximately 2,500 species of plants are used for medicinal purposes.[20-22] The current study was undertaken in the tribal region of Telangana state to list out the plant species having antidiabetic activity used by the traditional practitioners.

MATERIALS AND METHODS

Plant Material

The fresh plant of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds were collected from rural Jaipur district, Rajasthan. The plant was identified and authenticated in the Department of Botany, Rajasthan University, Jaipur, Rajasthan, India, (RBUL211697; RBUL211698; RBUL211699). The plant material was cleaned. The parts of the plant were shade dried and were coarsely powdered using a mechanical grinder. The powdered samples were stored in airtight and light-resistant containers to be used for further analyses.

Preparation of Plant Extract for Antidiabetic Studies[23,24]

- The *S. auriculata* leaves were shade dried at room temperature, and the dried leaves were powdered in a Wiley mill. A hundred grams of powdered *S. auriculata* leaves were packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to a qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract was used for antidiabetic studies.
- The *P. emblica* L. fruits were shade dried at room temperature, and the dried leaves were powdered in a Wiley mill. A hundred grams of powdered *P. emblica* L. fruits were packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to a qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract was used for antidiabetic studies.
- The *S. cumini* (L.) Skeels seeds were shade dried at room temperature, and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *S. cumini* (L.) Skeels seeds were packed in a Soxhlet apparatus and extracted with ethanol. The extract was subjected to a qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract was used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180–240 grams) were housed under standard environmental conditions at temperature (25 ± 2ºC), and light and dark (12:12 hours). Rats were fed with standard pellet diet (Kisan Feeds, New Delhi, India) and water *ad libitum*. The experimental protocol have been approved by the Institutional Animal Ethical Committee, Arya College of Pharmacy S-40, RIICO Industrial Area, Delhi Road Kukas, Jaipur, Rajasthan, India. CPCSEA No. 1013/PO/c/06/CPCSEA.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n = 6) of either sex selected by random sampling were used for acute toxicity study (acute oral toxicity-acute toxic class method. OECD. Paris. 2002). The animals were kept fasting overnight and provided only with water, after which the extracts were administered orally at 5 mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100, and 1,000 mg/kg body weight.

Induction of Experimental Diabetes[25]

Rats were induced diabetes by the administration of a simple intraperitoneal dose of alloxan monohydrate (150 mg/kg). Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with a blood glucose level of 200 to 260 mg/100 mL were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

In the investigation, a total of 42 rats (36 diabetic surviving rats and 6 normal rats) were taken and divided into 7 groups of 6 rats each.

Group I: Normal, untreated rats
Group II: Diabetic control rats
Group III: Diabetic rats given standard drug glibenclamide (100 mg/kg of body weight)
Group IV: Diabetic rats given ethanol extract of *S. auriculata* leaf (150 mg/kg of body weight)
Group V: Diabetic rats given ethanol extract of *P. emblica* L. fruit. (150 mg/kg of body weight)
Group VI: Diabetic rats given ethanol extract of *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight)
Group VI: Diabetic rats given combination of ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight)

Drug treatment was carried for 6 weeks with the help of an oral catheter everyday morning. At the end of drug treatment duration, all the animals were fasted overnight but allowed free access to water. Following morning, the following parameters were analyzed in blood and urine.

Collection of Blood Sample and Urine

At the end of drug treatment, all the animals were kept in metabolic cages for 24 hours. All the animals were fasted overnight but allowed free access to water. The next day morning, a blood sample was withdrawn by retro-orbital puncture under mild ether anesthesia.

Serum: Blood sample was collected into an Eppendorf tube. The sample was allowed to clot completely (20 minutes) before centrifugation. It was centrifuged at 4,000 rpm for 30 minutes in a refrigerated centrifuge at 4ºC. The serum separated as straw-colored supernatant was analyzed for the above stated biochemical parameters and markers. The serum was stored

at -20ºC until the completion of analysis.

Collection of urine sample: At the end of drug treatment, all the animals were kept in metabolic cages for 24 hours. Animals were fasted but allowed free access to water. Urine samples were collected after 24 hours in urine collecting bottles.

Biochemical Analysis

The animals were sacrificed at the end of the experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3,000 g for 10 minutes. The following parameters of diabetic complications (cardiomyopathy and nephropathy) analyzed in the normal, diabetic induced, and drug-treated rats.

Biochemical Estimation of Parameters of Diabetic Nephropathy

Measurement of renal function and biochemical parameters:

- Blood glucose was measured by Accu-Chek Active glucose strips. The blood glucose estimation was done weekly after the administration of the test compound.
- Protein estimation in urine and serum: The rat's urine was collected through an activity cage. The protein was precipitated with trichloroacetic acid (final concentration was 0.33 mol/liter). After mixtures had stood for 30 minutes at room temperature, the precipitates were centrifuged for 20 minutes at 110 xg. The precipitate was processed and, after reaction with biuret reagent, absorbency was measured by the colorimeter.

  The total protein concentration was determined by:

$$\text{Total Protein Concentration (g/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 6.5$$

  The formula was used for both determinations of protein in serum as well as in urine samples.
- Serum and urine albumin levels[26]: Bromo cresol green (BCG) method using Span and Ranbaxy diagnostic kits by an autoanalyser (Echo, Logotech Pvt. Ltd, India).

  Principle: Albumin binds with the dye Bromocresol Green in a buffered medium to form a green-colored complex. The intensity of the color formed is directly proportional to the amount of albumin present in the sample.

  Wavelength/filter: 630 nm (Hg 623 nm)/red

  Temperature: R. T.

  Lightpath: 1 cm

  Reagents: All chemicals must be AnalaR grade.

  Sodium hydroxide (NaOH) 1 M: Weigh out 4.0 g of sodium hydroxide (NaOH), dissolve, and makeup to 100 mL with distilled water. This solution is stable for several months at room temperature (25–35ºC) in a polypropylene container.

  Brij-35 30 g/dL: Readily available at the above concentration from S. D. Fine Chemicals or Loba Chemical Company in India. Solid Brij can also be obtained from Sigma Co. In this case, warm 30 grams solid Brij in a beaker in a small volume of distilled water to dissolve and make up to 100 mL with distilled water.

  Bromo cresol green (BCG) dye solution: Transfer 25 mL of 1 M NaOH into a 1-liter volumetric flask containing 600 NaOH distilled water. Add 5.6 g succinic acid and then add 56 mg of BCG powder. Mix and then make up to 1-liter with distilled water. Check the pH. If it is less than 4.15, adjust to 4.15 + 0.05 by the dropwise addition of 1 M NaOH.

  Add 100 mg sodium azide and 3.5 mL 30 g/dL Brij-35 to the reagent. Check the absorbance of the reagent at 630 nm/red filter against distilled water. It should be less than 0.2. If it is greater than 0.2, add some more Brij to bring down the absorbance. Store in a polyethylene container. Stable for 6 months at room temperature (25–35ºC).

  Standard: Bovine serum albumin: 4g/dL

  Procedure: The protocol of the procedure is described as follows. Mix all tubes well. Incubate at room temperature (25–35ºC) for 10 minutes. Set the spectrophotometer/filter photometer to zero using blank at 630 nm/red filter and measure the absorbance of standards, test.
- BUN value was measured by the BUN GLDH kit (Bhat Biotech Pvt.Ltd, Bangalore, India) technique as per instructions of manufacturers provided in BUN kits.

  A BUN test measures the amount of nitrogen in your blood that comes from the waste product urea. Urea is made when protein is broken down in your body. Urea is made in the liver and passed out of your body in the urine.

  A BUN test is done to see how well your kidneys are working. If your kidneys are not able to remove urea from the blood normally, your BUN level rises. Heart failure, dehydration, or a diet high in protein can also make your BUN level higher. Liver disease or damage can lower your BUN level. A low BUN level can normally occur in the second or third trimester of pregnancy.
- Serum creatinine rate was measured using the creatinine Kit by Mod. Jaffe's kinetic method (Coral Clinical System, Goa, India). Creatinine is the catabolic product of creatinine phosphate, which is used by the skeletal muscle. The daily production depends on the muscular mass, and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, and congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy.

  Principle: Picric acid in an alkaline medium reacts with creatinine to form an orange-colored complex with the alkaline picrate. The intensity of the color formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

  Creatinine + Alkaline picrate - Orange colored complex
- Serum and urine myoglobin estimation[27]: In the clinical methods for the quantitative estimation of serum proteins, filtration, through filter paper, is the usual procedure for the separation of the globulin precipitated from albumin by a 1.50 M sodium sulfate solution. On account of the nature of the precipitate, a highly retentive paper is needed, and also, with most sera, the filtrate must be refiltered many times before it is clear. Paper adsorbs a definite amount of

the soluble protein. Therefore, it is necessary to discard the first portion of the filtrate because there is a loss of albumin. Later portions are uniform in nitrogen concentration and contain the protein that is soluble in this salt concentration.

Histopathological studies were also carried out of the heart to evaluate the degree of beneficiary effect of new pharmacological interventions by seeing morphometric changes. Tissues were also analyzed for degree of deterioration by loss of contractile protein, necrosis, inflammation, myocytolysis, and contracture bands.

Histopathological Examination

At the end of the experiments, all rats were sacrificed, and pathological analysis of the heart and kidney was performed. The kidney tissues were preserved in buffered neutral formalin and stored at -20ºC until processed for histopathology. Tissues were preserved in 1% w/v glutaraldehyde 4% w/v formaldehyde in phosphate buffer, pH 7.2 at 4ºC until processed for electron microscopy. Tissues were processed for histopathology at room temperature and involved the following steps: (a) fixation, (b) processing of tissues—dehydrating, clearing, and embedding, (c) preparation and cutting of sections, and (d) attaching sections to slides. After processing, sections were stained using hematoxylin-eosin stain using Harris's alum hematoxylin and stock 1% w/v alcohol eosin solution. The stained sections were finally mounted in DPX.

Statistical Analysis

Statistical analysis was performed with graph SYSTAT 12 software. Quantitative data were expressed as mean ± standard error mean (SEM); all data were statistically analyzed by student's t test. The statistically significant difference between groups was set at $p < 0.05$.

RESULTS

Effect of Drugs in Diabetic Rats on Body Weight

The body weight decreased rapidly in alloxan-induced diabetes in rats. Measurement of body weight of the rats of all experimental groups is shown in Table 1 and Figure 1. The body weight increased normally in control rats, while alloxan-induced diabetic rats (negative control) showed a significant decrease in body weight as soon as 1-week post alloxan injection (pre: 167.41 ± 4.958 to 162.62 ± 5.78, $p < 0.01$). A progressive loss of body weight was noted after 14 days in the negative control group (pre: 167.41 ± 4.958 to 156.89 ± 6.203, $p < 0.001$). The maximum decrease in body weight was observed after 6 weeks of alloxan injection (pre: 167.41 ± 4.958 to 129.03 ± 5.932, $p < 0.001$). The weight of the animals of other groups was also decreased significantly until day 21 as compared to the negative control group (Table 1). The individual extracts [*Senna (cassia) auriculata (S. auriculata)*, *P. emblica* L., and *S. cumini* (L.) Skeels] and standard drug-treated diabetic rats showed a non-significant decrease in body weight, whereas the combination of extracts group showed a non-significant increase in body weight (i.e., no weight gain).

Effect of Drugs in Diabetic Rats on Blood Glucose Level

The blood glucose level of all experimental groups, except the normal control group, was increased significantly after the alloxan injection till day 21 (Table 2; Figure 2). On day 28 to 42 of diabetes induction, the diabetic group observed with a significant increase in blood glucose levels from normal control animals ($p < 0.001$). In the diabetic group (negative control), the blood glucose level increased to the maximum measurable value of 369.36 ± 7.784 mg/dL on day 42 and found to be significantly increased ($p < 0.001$) compared to the value of day 0 was 84.42 ± 6.384 mg/dL. In control, animals

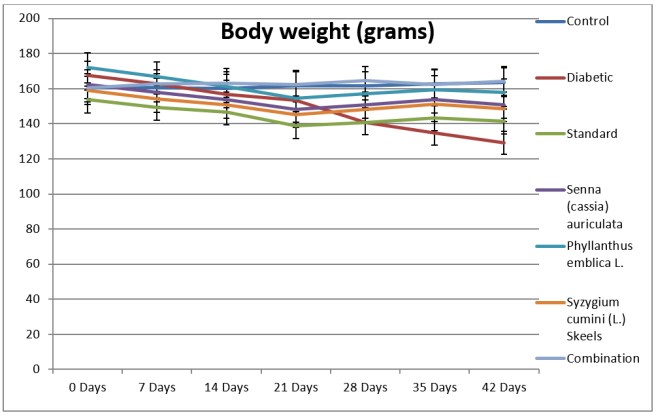

Figure 1: The effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on body weight in alloxan-induced diabetes in rats

Table 1: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on body weight in rats (in grams)

Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	160.62 ± 6.412	160.32 ± 6.354	160.24 ± 6.38	161.48 ± 5.833	161.62 ± 4.655	162.81 ± 4.83	163.64 ± 5.832
Diabetic	167.41 ± 4.958	162.62 ± 5.78	156.89 ± 6.203	153.44 ± 6.662	140.69 ± 5.894	134.64 ± 6.365	129.03 ± 5.932
Standard	153.83 ± 5.132	149.47 ± 6.283	146.61 ± 6.051	138.68 ± 6.452	140.84 ± 5.966	143.23 ± 5.734	141.27 ± 6.057
S. auriculata	162.47 ± 5.334	157.83 ± 6.125	153.89 ± 4.894	148.23 ± 5.483	150.69 ± 5.746	153.95 ± 6.127	150.67 ± 4.893
P. emblica L.	171.93 ± 6.052	166.79 ± 5.943	161.42 ± 6.423	154.46 ± 5.651	157.03 ± 6.198	159.28 ± 6.235	157.81 ± 5.846
S. cumini (L.) Skeels	159.06 ± 6.429	154.21 ± 5.841	150.71 ± 6.236	145.25 ± 6.374	148.26 ± 5.784	151.22 ± 6.063	148.62 ± 5.734
Combination	160.56 ± 6.274	162.69 ± 6.458	163.18 ± 5.732	162.36 ± 5.841	164.47 ± 6.588	162.54 ± 5.852	164.38 ± 5.942

Values are expressed mean ± SEM; n = 6; ** = $p < 0.01$; *** = $p < 0.001$, when compared to normal control group; b = ns, when compared to normal control group; a*** = $p < 0.001$, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 2: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood glucose level in rats (in mg/dL)

Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	85.83 ± 6.428	85.82 ± 7.042	85.68 ± 6.893	85.46 ± 7.631	85.24 ± 7.924	84.81 ± 6.725	85.87 ± 7.146
Diabetic	84.42 ± 6.384	272.79 ± 8.034	321.45 ± 7.782	328.22 ± 6.883	331.08 ± 7.864	350.52 ± 8.031	369.36 ± 7.784
Standard	87.07 ± 6.836	256.52 ± 7.428	319.56 ± 6.942	204.97 ± 7.147	154.72 ± 6.842	127.94 ± 7.253	96.86 ± 6.631
S. auriculata	88.72 ± 7.035	259.98 ± 6.931	325.92 ± 6.739	332.04 ± 6.853	211.05 ± 7.326	141.81 ± 7.736	124.42 ± 7.042
P. emblica L.	86.41 ± 7.482	264.63 ± 7.156	323.17 ± 7.035	368.98 ± 7.452	209.43 ± 6.883	133.06 ± 6.903	112.07 ± 6.942
S. cumini (L.) Skeels	87.69 ± 6.832	262.44 ± 6.582	324.85 ± 6.832	365.37 ± 6.894	212.08 ± 7.237	142.87 ± 7.287	126.25 ± 7.051
Combination	88.21 ± 6.674	253.94 ± 6.745	326.63 ± 8.052	182.49 ± 7.312	156.27 ± 6.548	132.83 ± 8.126	98.83 ± 6.932

Q9 Values are expressed mean ± SEM; n = 6; ** = p < 0.01; *** = p < 0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 3A: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood protein level in rats (in mg/dL)

Serum protein (g/dL)

Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	7.42 ± 0.044	7.42 ± 0.039	7.48 ± 0.052	7.46 ± 0.037	7.46 ± 0.051	7.44 ± 0.037	7.46 ± 0.043
Diabetic	7.48 ± 0.051	14.48 ± 0.062	15.98 ± 0.043	17.76 ± 0.038	20.58 ± 0.043	25.38 ± 0.052	25.18 ± 0.046
Standard	7.32 ± 0.052	13.53 ± 0.058	12.98 ± 0.041	10.86 ± 0.047	7.38 ± 0.052	7.92 ± 0.056	7.42 ± 0.039
S. auriculata	7.34 ± 0.038	16.52 ± 0.052	13.98 ± 0.044	15.82 ± 0.041	8.16 ± 0.047	7.85 ± 0.053	7.98 ± 0.039
P. emblica L.	7.36 ± 0.045	16.48 ± 0.048	15.92 ± 0.036	15.76 ± 0.052	8.18 ± 0.043	7.94 ± 0.044	8.02 ± 0.053
S. cumini (L.) Skeels	7.67 ± 0.052	16.68 ± 0.043	15.98 ± 0.038	15.98 ± 0.051	8.23 ± 0.037	7.87 ± 0.046	8.06 ± 0.039
Combination	7.43 ± 0.051	16.35 ± 0.036	14.02 ± 0.042	12.83 ± 0.035	8.21 ± 0.046	7.85 ± 0.042	7.48 ± 0.045

Q9 Values are expressed mean ± SEM; n = 6; ** = p < 0.01; *** = p < 0.0001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.0001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

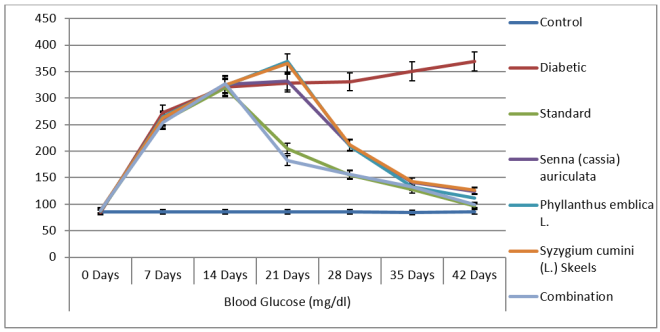

Figure 2: The effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood glucose level (mg/dL) in alloxan-induced diabetes in rats

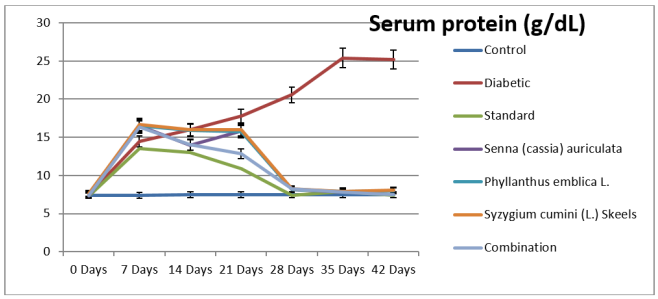

Figure 3A: The effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood protein levels in rats (in mg/dL) in alloxan-induced diabetic rats

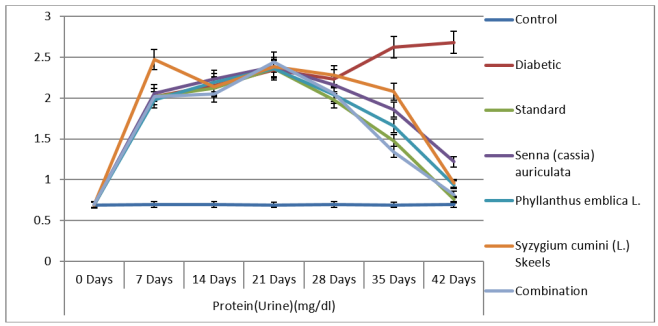

Figure 3B: The effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on protein level in urine in alloxan-induced diabetic rats

remain normoglycaemic during the entire testing period of Q5 42 days (Table 5.2). The animals treated on the day 21st with different groups of drug therapy like standard and extracts of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and it was observed that a significant decrease in blood glucose level ($p < 0.001$) compared to the normal control group on day 28, 35, and 42.

Effect of Drugs in Diabetic Rats on Protein Level in Blood and in Urine

The protein level in blood in all experimental groups, except the normal control group, was significantly increased, and in urine, protein excretion rate is increased after alloxan injection (Tables 3A and B; Figures 3A and B). On the 21, 28, and 35 of diabetes induction, the negative control (diabetic) group with a statistically significant increase in blood protein level and increased in urine protein level from the control group ($p < 0.001$). In diabetic group (negative control group), the blood protein level increased to the maximum value of 7.48 ± 0.051 to 25.18 ± 0.046 mg/dL and urine protein level increased 0.692 ± 0.061 to 2.68 ± 0.056, and found to have statistical significance

Table 3B: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on protein level in urine in rats (mg/dL)

Protein (urine) (mg/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	0.694 ± 0.052	0.696 ± 0.047	0.696 ± 0.039	0.694 ± 0.042	0.696 ± 0.036	0.694 ± 0.051	0.698 ± 0.055
Diabetic	0.692 ± 0.061	2.02 ± 0.052	2.15 ± 0.041	2.34 ± 0.053	2.234 ± 0.046	2.62 ± 0.061	2.68 ± 0.056
Standard	0.692 ± 0.064	2.02 ± 0.063	2.12 ± 0.058	2.36 ± 0.049	1.98 ± 0.052	1.48 ± 0.048	0.76 ± 0.063
S. auriculata	0.692 ± 0.052	2.06 ± 0.063	2.23 ± 0.061	2.38 ± 0.059	2.16 ± 0.049	1.86 ± 0.057	1.22 ± 0.058
P. emblica L.	0.692 ± 0.045	1.98 ± 0.062	2.19 ± 0.056	2.36 ± 0.064	2.04 ± 0.054	1.663 ± 0.062	0.94 ± 0.049
S. cumini (L.) Skeels	0.694 ± 0.049	2.47 ± 0.053	2.14 ± 0.048	2.38 ± 0.069	2.28 ± 0.043	2.08 ± 0.052	0.96 ± 0.056
Combination	0.697 ± 0.043	2.02 ± 0.054	2.05 ± 0.049	2.44 ± 0.058	2.06 ± 0.055	1.34 ± 0.051	0.82 ± 0.062

Q9 Values are expressed mean ± SEM; n = 6; ** = p <0.01; *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 4A: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on serum albumin (g/dL)

Serum albumin (mg/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	1.98 ± 0.037	1.96 ± 0.043	1.94 ± 0.031	1.92 ± 0.042	1.9 ± 0.036	1.88 ± 0.038	1.94 ± 0.042
Diabetic	1.94 ± 0.043	1.12 ± 0.033	1.04 ± 0.035	0.98 ± 0.042	0.86 ± 0.038	0.76 ± 0.032	0.74 ± 0.043
Standard	1.92 ± 0.032	0.98 ± 0.041	1.22 ± 0.046	1.42 ± 0.031	1.64 ± 0.035	1.78 ± 0.035	1.86 ± 0.042
S. auriculata	1.98 ± 0.043	1.17 ± 0.038	1.18 ± 0.037	1.38 ± 0.038	1.66 ± 0.039	1.54 ± 0.037	1.64 ± 0.033
P. emblica L.	1.88 ± 0.039	1.12 ± 0.039	1.28 ± 0.033	1.38 ± 0.041	1.62 ± 0.038	1.72 ± 0.041	1.82 ± 0.036
S. cumini (L.) Skeels	1.96 ± 0.042	1.19 ± 0.043	1.18 ± 0.034	1.28 ± 0.036	1.58 ± 0.045	1.62 ± 0.043	1.87 ± 0.044
Combination	1.98 ± 0.038	1.06 ± 0.038	1.26 ± 0.045	1.35 ± 0.043	1.62 ± 0.038	1.86 ± 0.039	1.96 ± 0.039

Q9 Values are expressed mean ± SEM; n = 6; ** = p <0.01; *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

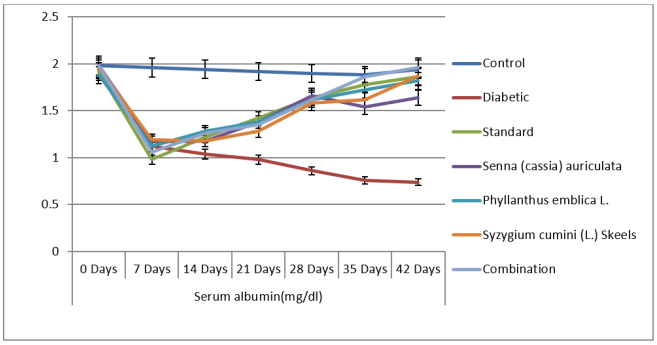

Figure 4B: The effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on serum albumin (mg/dL) in alloxan-induced diabetic rats

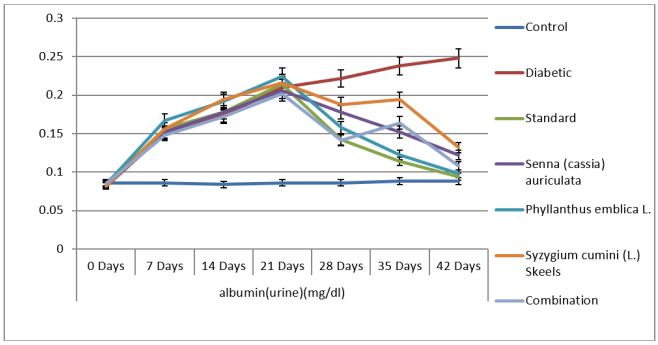

Figure 4B: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on albumin urine (mg/dL); values are expressed mean ± SEM; n = 6, ** = p <0.01, *** = p <0.001, when compared to normal control group; b = ns when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns when compared to standard group; standard = glimipirides Q9

(p <0.001). In contrast, the control group shows normal protein levels in the blood (7.42 ± 0.044 to 7.46 ± 0.043) during the entire testing period of 42 days (Tables 5A and B; Figures 5A and B). Q6 The animal treated with *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination were observed with a significant decrease in blood protein level and urine protein level (p <0.001) compared to the negative control group on day 21st, 28th, 35th, and 42nd. The blood protein level in combination therapy on day 42nd was 7.48 ± 0.045, which was significant compared with the negative control (diabetic) group.

Effect of Drugs in Diabetic Rats on Albumin (mg/dL) level in Blood and in Urine

The albumin level in blood in all experimental groups, except normal control group, was significantly increased, and in urine, albumin excretion rate is increased after alloxan injection (Tables 4A and B; Figures 4A and B). On the 21, 28, and 35 Q7 of diabetes induction, the negative control (diabetic) group with a statistically significant decrease in blood albumin level and increased urine albumin level from the control group (p <0.001). In diabetic group (negative control group) the blood albumin level decreased to the maximum value of 1.94 ± 0.043 to 0.248 ± 0.007 mg/dL, and urine albumin level increased 0.082 ± 0.009 to 2.68 ± 0.056 and found to be statistical significance (p <0.001). In contrast, the control group shows normal albumin levels in the blood (1.98 ± 0.037 to 1.94 ± 0.042) during the entire testing period of 42 days

Table 4B: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on albumin urine (mg/dL)

Albumin (urine) (mg/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	0.086 ± 0.008	0.086 ± 0.009	0.084 ± 0.007	0.086 ± 0.004	0.086 ± 0.013	0.088 ± 0.013	0.088 ± 0.012
Diabetic	0.082 ± 0.009	0.152 ± 0.012	0.174 ± 0.013	0.213 ± 0.009	0.222 ± 0.008	0.238 ± 0.012	0.248 ± 0.007
Standard	0.083 ± 0.012	0.156 ± 0.008	0.178 ± 0.007	0.214 ± 0.012	0.142 ± 0.009	0.114 ± 0.011	0.094 ± 0.012
S. auriculata	0.086 ± 0.008	0.152 ± 0.007	0.178 ± 0.009	0.206 ± 0.011	0.178 ± 0.010	0.152 ± 0.007	0.122 ± 0.008
P. emblica L.	0.084 ± 0.012	0.167 ± 0.014	0.192 ± 0.006	0.224 ± 0.007	0.158 ± 0.0011	0.122 ± 0.006	0.098 ± 0.007
S. cumini (L.) Skeels	0.082 ± 0.013	0.156 ± 0.009	0.194 ± 0.012	0.216 ± 0.008	0.188 ± 0.013	0.194 ± 0.011	0.132 ± 0.009
Combination	0.085 ± 0.010	0.148 ± 0.008	0.172 ± 0.012	0.202 ± 0.009	0.141 ± 0.011	0.164 ± 0.007	0.108 ± 0.011

Q9 Values are expressed mean ± SEM; n = 6; ** = p < 0.01; *** = p < 0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

(Tables 4A and B; Figures 4A and B). The animal treated with *S. auriculata, P. emblica* L., and *S. cumini* (L.) Skeels and its combination were observed with significant normal blood albumin levels and urine albumin levels ($p < 0.001$) compared to the negative control group on day 21st, 28th, 35th, and 42nd. The blood albumin level in combination therapy on day 42nd was 1.96 ± 0.039, which was significant compared with the negative control (diabetic) group.

Effect of Drugs in Diabetic Rats on Myoglobin Level in Blood (ng/dL) and Urine (mg/dL)

Myoglobin levels are indications of diabetic cardiomyopathy. The myoglobin level in blood in all experimental groups, except the normal control group, was significantly increased, and in urine, myoglobin excretion rate is increased after alloxan injection (Tables 5A and B; Figures 5A and B). On the 21, 28, and 35 of diabetes induction, the negative control (diabetic) group with a statistically significant increase in blood myoglobin level and increased in urine myoglobin level from the control group ($p < 0.001$). In diabetic group (negative control group) the blood myoglobin level increased to the maximum value of 0.042 ± 0.00274 to 0.056 ± 0.00207 ng/dL and urine myoglobin level increased 0.0048 ± 0.00142 to 0.0098 ± 0.00107 mg/dL and found to be statistically significant ($p < 0.001$). In contrast, the

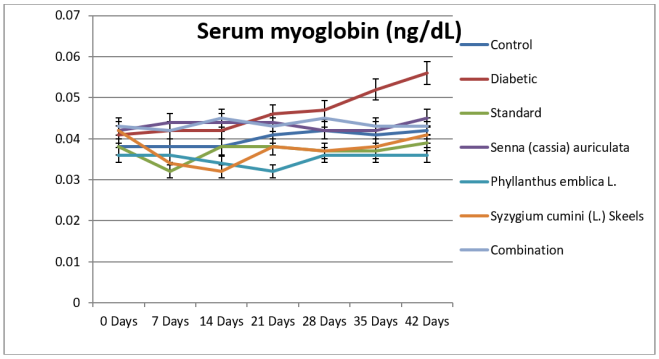

Figure 5A: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on myoglobin serum (ng/dL); values are expressed mean ± SEM; n = 6, ** = p < 0.01, *** = p < 0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipirides

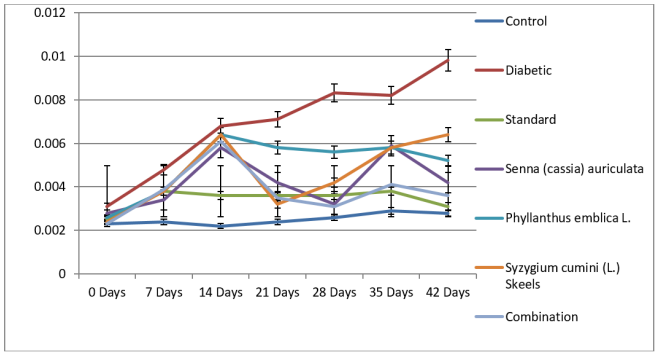

Figure 5B: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on myoglobin serum (ng/dL); values are expressed mean ± SEM; n = 6, ** = p < 0.01, *** = p < 0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 5A: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on myoglobin serum (ng/dL)

Serum myoglobin (ng/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	0.038 ± 0.00238	0.038 ± 0.00219	0.038 ± 0.00249	0.041 ± 0.00310	0.042 ± 0.00238	0.041 ± 0.00192	0.042 ± 0.00276
Diabetic	0.041 ± 0.00210	0.042 ± 0.00274	0.042 ± 0.00182	0.046 ± 0.00241	0.047 ± 0.00239	0.052 ± 0.00169	0.056 ± 0.00207
Standard	0.038 ± 0.00186	0.032 ± 0.00215	0.038 ± 0.00236	0.038 ± 0.00183	0.037 ± 0.00206	0.037 ± 0.00294	0.039 ± 0.00219
S. auriculata	0.042 ± 0.00193	0.044 ± 0.00223	0.044 ± 0.00193	0.044 ± 0.00266	0.042 ± 0.00184	0.042 ± 0.00213	0.045 ± 0.00189
P. emblica L.	0.036 ± 0.00273	0.036 ± 0.00169	0.034 ± 0.00235	0.032 ± 0.00186	0.036 ± 0.00212	0.036 ± 0.00255	0.036 ± 0.00177
S. cumini (L.) Skeels	0.042 ± 0.00403	0.034 ± 0.00208	0.032 ± 0.00241	0.038 ± 0.00193	0.037 ± 0.00213	0.038 ± 0.00158	0.041 ± 0.00223
Combination	0.043 ± 0.00257	0.042 ± 0.00241	0.045 ± 0.00183	0.043 ± 0.00199	0.045 ± 0.00225	0.043 ± 0.00214	0.043 ± 0.00175

Q9 Values are expressed mean ± SEM; n = 6; ** = p < 0.01; *** = p < 0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p < 0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 5B: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on myoglobin serum (ng/dL)

Myoglobin (urine) (mg/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	0.0023 ± 0.00142	0.0024 ± 0.00131	0.0022 ± 0.00129	0.0024 ± 0.00106	0.0026 ± 0.00118	0.0029 ± 0.00108	0.0028 ± 0.00119
Diabetic	0.0031 ± 0.00148	0.0048 ± 0.00142	0.0068 ± 0.00127	0.0071 ± 0.00124	0.0831 ± 0.00106	0.0082 ± 0.00120	0.0098 ± 0.00107
Standard	0.0025 ± 0.00152	0.0038 ± 0.00139	0.0036 ± 0.00122	0.0036 ± 0.00136	0.0036 ± 0.00104	0.0038 ± 0.00142	0.0031 ± 0.00134
S. auriculata	0.0028 ± 0.00128	0.0034 ± 0.00125	0.0058 ± 0.00137	0.0042 ± 0.00128	0.0032 ± 0.00126	0.0059 ± 0.00141	0.0042 ± 0.00129
P. emblica L.	0.0026 ± 0.00126	0.0038 ± 0.00134	0.0064 ± 0.00153	0.0058 ± 0.00142	0.0056 ± 0.00148	0.0058 ± 0.00108	0.0052 ± 0.00119
S. cumini (L.) Skeels	0.0024 ± 0.00147	0.0038 ± 0.00129	0.0064 ± 0.00125	0.0032 ± 0.00139	0.0042 ± 0.00134	0.0058 ± 0.00130	0.0064 ± 0.00126
Combination	0.0023 ± 0.00136	0.0039 ± 0.00134	0.0061 ± 0.00152	0.0035 ± 0.00124	0.0031 ± 0.00128	0.0041 ± 0.00132	0.0036 ± 0.00125

Values are expressed mean ± SEM; n = 6; ** = p <0.01; *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

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control group shows normal myoglobin level in the blood (0.038 ± 0.00238 to 0.042 ± 0.00276) during the entire testing period of 42 days (Tables 5A and B; Figures 5A and B). The animal treated with *S. auriculata*, *P. emblica* L. and *S. cumini* (L.) Skeels and its combination were observed with significant normal blood myoglobin level and urine myoglobin level ($p < 0.001$) compared to the negative control group on day 21st, 28th, 35th, and 42nd. The blood myoglobin level in combination therapy on day 42nd was 0.043 ± 0.00175, which was significant compared with the negative control (diabetic) group.

Effect of Drugs in Diabetic Rats on Blood Urea Nitrogen (BUN) (mg/dL)

The BUN (mg/dL) level in blood is indicated in diabetic nephropathy. BUN (mg/dL) level in blood in all experimental groups, except the normal control group, was significantly increased after alloxan injection (Table 6; Figure 6). On the 21, 28, and 35 of diabetes induction, the negative control (diabetic) group with a statistically significant increase in BUN ($p < 0.001$). In the diabetic group (negative control group), the BUN increased to the maximum value of 23.04 ± 1.093 to 124.81 ± 1.238 mg/dL and found to be statistical significance ($p < 0.001$). In contrast, the control group shows a normal BUN level in the blood (22.76 ± 1.352 to 24.04 ± 1.246) during the entire testing period of 42 days (Table 6; Figure 6). The animal treated with *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination were observed with a significant normal BUN level ($p < 0.001$) compared to the negative control group on day 21st, 28th, 35th, and 42nd. The BUN level in combination therapy on day 42nd was 29.03 ± 1.229, which was significant compared with the negative control (diabetic) group.

Effect of Drugs in Diabetic Rats on Serum Creatinine (µmol/dL)

Serum creatinine (µmol/dL) level in blood is indicated in diabetic nephropathy. The serum creatinine (µmol/dL) level in blood in all experimental groups, except the normal control group, was significantly increased after alloxan injection

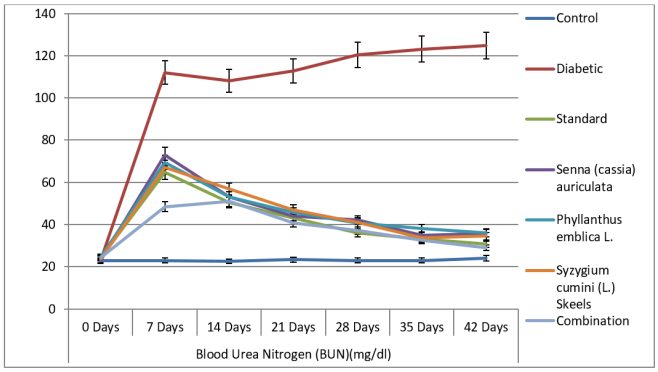

Figure 6: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood urea nitrogen (BUN) (mg/dL); values are expressed mean ± SEM; n = 6, ** = p <0.01, *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipirides

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Table 6: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on blood urea nitrogen (BUN) (mg/dL)

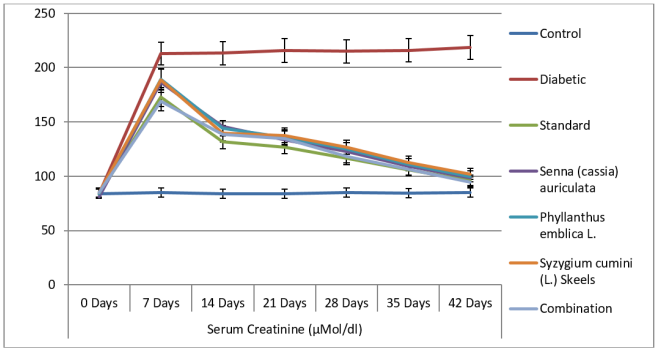
Blood urea nitrogen (BUN) (mg/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	22.76 ± 1.352	23.02 ± 1.043	22.57 ± 0.937	23.41 ± 1.042	22.94 ± 1.093	23.02 ± 1.129	24.04 ± 1.246
Diabetic	23.04 ± 1.093	111.98 ± 0.951	108.09 ± 1.303	112.75 ± 1.205	120.47 ± 1.047	123.06 ± 0.859	124.81 ± 1.238
Standard	24.31 ± 0.936	64.73 ± 1.238	50.36 ± 1.152	43.06 ± 1.243	36.09 ± 0.981	33.06 ± 1.204	30.64 ± 1.263
S. auriculata	23.08 ± 1.149	72.93 ± 1.146	53.03 ± 1.142	44.07 ± 1.041	42.07 ± 1.192	34.82 ± 1.206	35.81 ± 1.186
P. emblica L.	24.63 ± 1.206	69.41 ± 1.072	52.94 ± 1.327	45.72 ± 1.24	40.88 ± 1.093	38.02 ± 0.941	36.06 ± 1.123
S. cumini (L.) Skeels	23.84 ± 1.125	67.08 ± 1.226	56.85 ± 1.082	46.91 ± 1.118	41.04 ± 0.894	33.63 ± 1.283	34.53 ± 1.177
Combination	24.45 ± 0.892	48.46 ± 1.173	50.92 ± 1.307	40.82 ± 1.262	37.09 ± 1.139	32.62 ± 1.284	29.03 ± 1.229

Q9 Values are expressed mean ± SEM; n = 6; ** = p <0.01; *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

Table 7: Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on serum creatinine (**µmol**/dL)

Serum creatinine (µmol/dL)							
Groups	*0 days*	*7 days*	*14 days*	*21 days*	*28 days*	*35 days*	*42 days*
Control	83.92 ± 5.926	84.87 ± 6.042	84.06 ± 7.231	83.85 ± 8.216	84.72 ± 6.732	84.62 ± 8.564	84.93 ± 5.936
Diabetic	84.06 ± 6.723	212.94 ± 5.472	213.45 ± 6.261	215.78 ± 7.319	214.94 ± 8.031	216.02 ± 5.932	218.56 ± 7.586
Standard	84.06 ± 6.674	172.93 ± 5.832	132.03 ± 7.341	126.92 ± 8.639	116.58 ± 6.455	105.83 ± 5.942	96.47 ± 5.908
S. auriculata	80.87 ± 7.041	186.52 ± 6.894	146.08 ± 5.834	133.43 ± 8.172	122.62 ± 6.846	108.94 ± 7.493	98.42 ± 5.526
P. emblica L.	83.62 ± 6.942	189.41 ± 8.172	144.09 ± 8.058	135.81 ± 6.905	124.76 ± 7.453	110.63 ± 6.639	99.73 ± 6.064
S. cumini (L.) Skeels	84.75 ± 5.723	188.95 ± 6.042	139.74 ± 5.745	137.64 ± 6.437	126.81 ± 7.043	112.77 ± 7.586	101.97 ± 6.052
Combination	84.74 ± 5.832	168.97 ± 6.493	138.38 ± 7.630	134.79 ± 5.842	118.36 ± 7.936	106.42 ± 5.946	94.83 ± 6.678

Q9 Values are expressed mean ± SEM; n = 6; ** = p <0.01; *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride



Q9 **Figure 7:** Effect of *S. auriculata*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination on serum creatinine (µmol/dL); values are expressed mean ± SEM; n=6, ** = p <0.01, *** = p <0.001, when compared to normal control group; b = ns, when compared to normal control group; a*** = p <0.001, when compared to negative control group; c = ns, when compared to standard group; standard = glimipiride

(Table 7; Figure 7). On the 21, 28, and 35 of diabetes induction, the negative control (diabetic) group with a statistically significant increase in serum creatinine (µmol/dL) (p <0.001). In diabetic group (negative control group) the serum creatinine (µmol/dL) increased to the maximum value of 84.06 ± 6.723 to 218.56 ± 7.586 (µmol/dL) and found to be statistical significance (p <0.001). In contrast, the control group shows normal serum creatinine (µmol/dL) level in the blood (83.92 ± 5.926 to 84.93 ± 5.936) during the entire testing period of 42 days (Table 7; Figure 7). The animal treated with *f*, *P. emblica* L., and *S. cumini* (L.) Skeels and its combination were observed with significant normal serum creatinine (µmol/dL) level (p <0.001) compared to the negative control group on day 21st, 28th, 35th, and 42nd. The serum creatinine (µmol/dL) level in combination therapy on day 42nd was 94.83 ± 6.678, which was significant compared with the negative control (diabetic) group.

DISCUSSION

The present work has detected the effect of ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination in alloxan-induced diabetic complications like nephropathy and cardiomyopathy in rats. Alloxan injection caused diabetic nephropathy and cardiomyopathy, probably due to the destruction of the β cells of islets of Langerhans of the pancreas, over the production of high blood glucose level and decreased utilization by tissues from the fundamental bases of hyperglycemia in *Diabetes mellitus*. Alloxan prevents DNA synthesis and also prevents cellular reproduction with a much smaller dose than that dose needed for inhibiting the substance concentration of DNA or inhibiting many of the enzymes involved in DNA synthesis. Hyperglycemia accompanied by weight loss was seen in adult rats treated with alloxan, which were stable for weeks, which indicates the irreversible destruction of β cells of islets of Langerhans of the pancreas. The STZ is most commonly used to induce diabetes Q3 in experimental animals because it is a simple, inexpensive, and available method.

Diabetic nephropathy and cardiomyopathy are a long-term complication of diabetes observed in 60 to 70% of all diabetic patients that develops early in the course of the disease.

Diabetic cardiomyopathy is demonstrated as there is a high degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MP), myocytolysis (MCL), necrosis (N), contracture bands (CB), or inflammation (I) in cardiomyocytes. Diabetic nephropathy is characterized by deterioration of kidney by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), the nodular lesion (NL) (Figures 9 to 21).

Diabetic nephropathy and cardiomyopathy are triggered by hyperglycemia, which leads to a persistent accelerated flux of glucose through the polyol pathway. The rate-limiting enzyme in this pathway is aldose reductase. The increased flux through the polyol pathway is followed by abnormal PKC metabolism, oxidative stress, accelerated glycation, and decreased endoneurial capillary perfusion, leading eventually to cardiac and nephron degeneration. The hypoglycemic effect was observed with the treatment of ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination in alloxan-induced hyperglycemic rats, with the maximum effect seen in the combination group, which may be due to its antidiabetic effect because all the three drugs are used in type 2 DM. Blood glucose is increased in a wide variety of diabetes if the elevation of blood glucose remains for a long time; cardiovascular diseases such as diabetic cardiomyopathy develop in diabetic patients. Ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination significantly

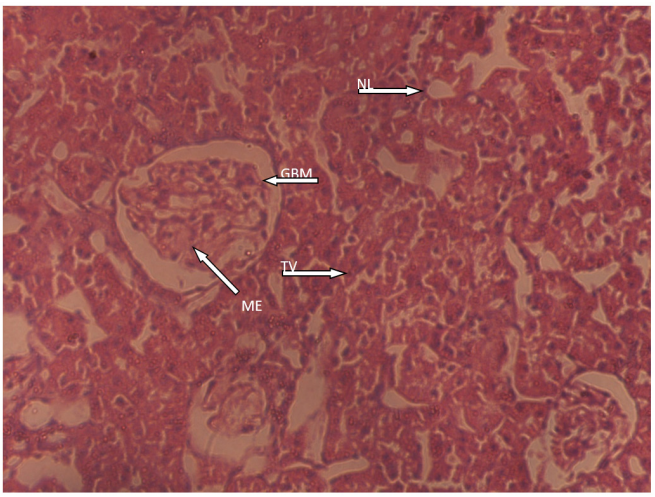


Figure 8: Photomicrograph of section of the normal control group (after 7 weeks' treatment); there is no degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), nodular lesion (NL)

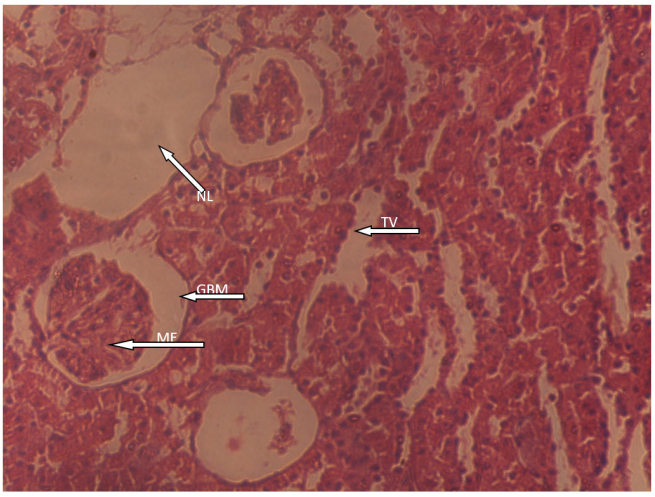


Figure 9: Photomicrograph of section of the diabetic control group showing nephropathy (after 7 weeks' treatment); there is high degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), nodular lesion (NL)

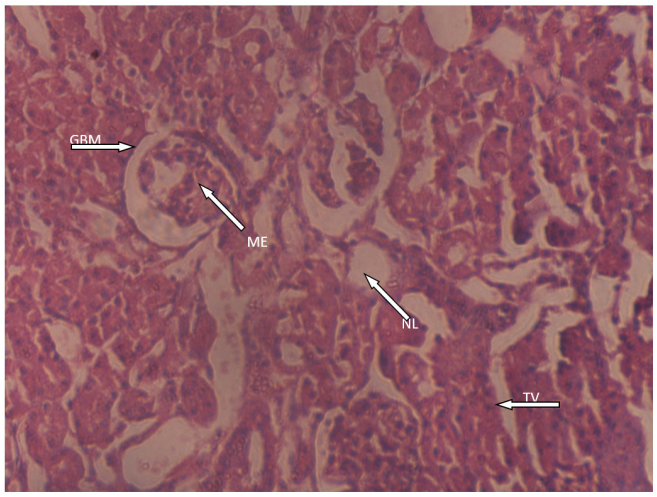


Figure 10: Photomicrograph of section of the standard group treated with glimepiride showing nephropathy (after 7 weeks' treatment); there is less degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expantion (ME), nodular lesion (NL), but not significant as like dual therapy

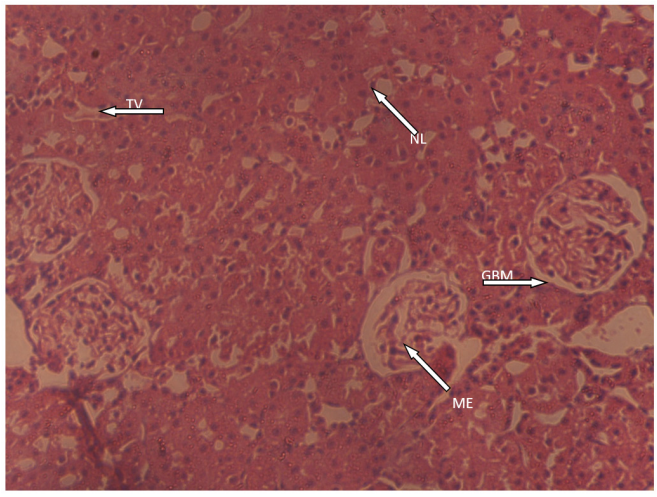


Figure 11: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of *S. auriculata* leaf (150 mg/kg of body weight) showing nephropathy (after 7 weeks' treatment); there is less degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expantion (ME), nodular lesion (NL)

reduced the blood glucose. Serum protein is reduced in a wide variety of type 2 diabetes, contributes considerably to increased risk of cardiovascular events. Serum protein was found to decrease significantly with a combination of partially significant with ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) in a single therapy.

Serum albumin is also reduced in diabetic cardiomyopathy group serum myoglobin is a biomarker for cardiovascular risk, is also reduced in diabetic cardiomyopathy groups. A combination is significantly effective to normalize serum myoglobin. Protein, albumin, and myoglobin in urine are also elevated in diabetic cardiomyopathy groups, ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels

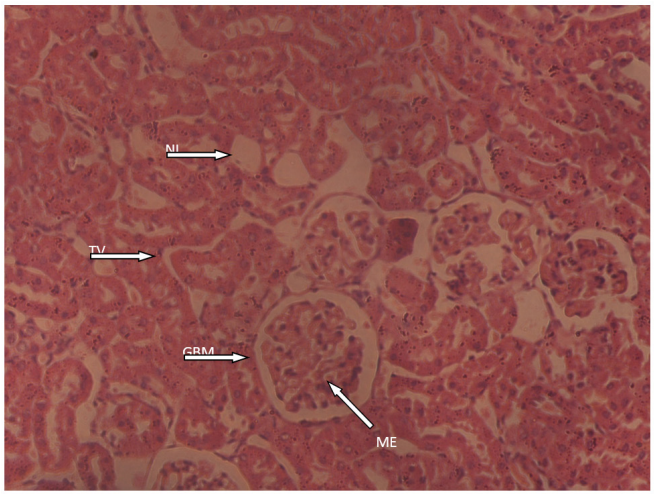


Figure 12: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of *P. emblica* L. fruit (150 mg/kg of body weight) showing nephropathy (after 7 weeks' treatment); there is less degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), nodular lesion (NL), but not significant as compared to dual therapy

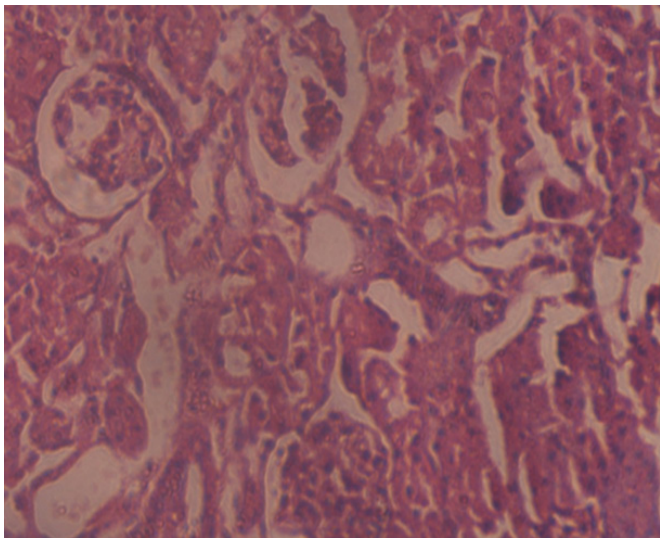


Figure 13: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks' treatment); there is less degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), nodular lesion (NL), but not significant as compared to dual therapy

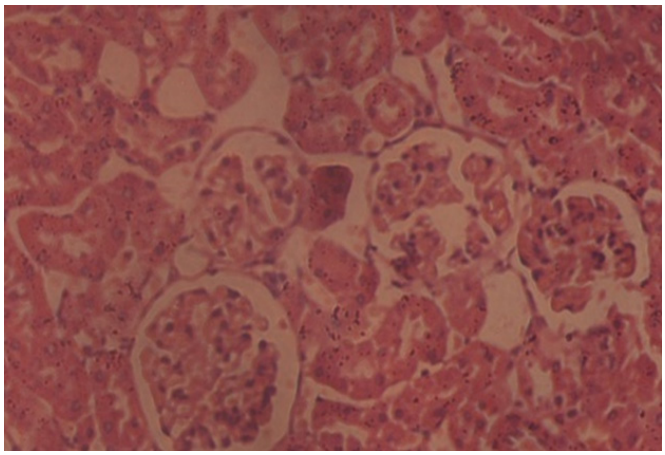


Figure 14: Photomicrograph of section of the diabetic treatment group, treated with combination of ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks' treatment); there is less degree of deterioration by tubular vacuolization (TV), thickening of the glomerular basement membrane, mesangial matrix expansion (ME), nodular lesion (NL), but not significant as compared to dual therapy

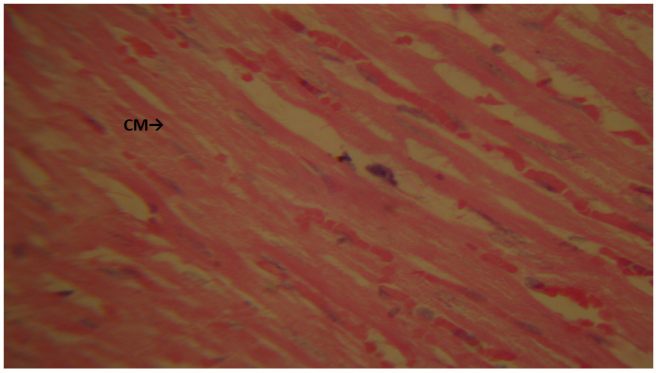


Figure 15: Photomicrograph of section of normal heart from rats in control group showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is no deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation (I)

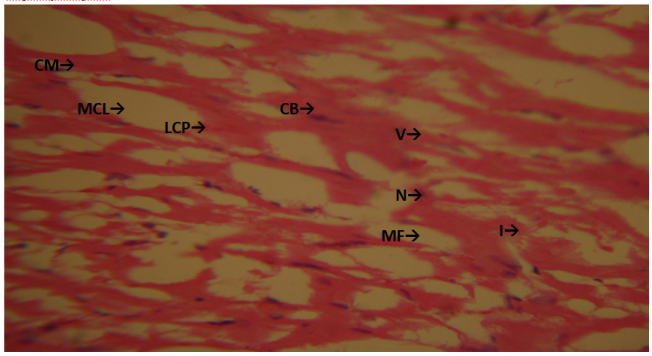


Figure 16: Photomicrograph of section of the diabetic heart group showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is high degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MP), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation (I)

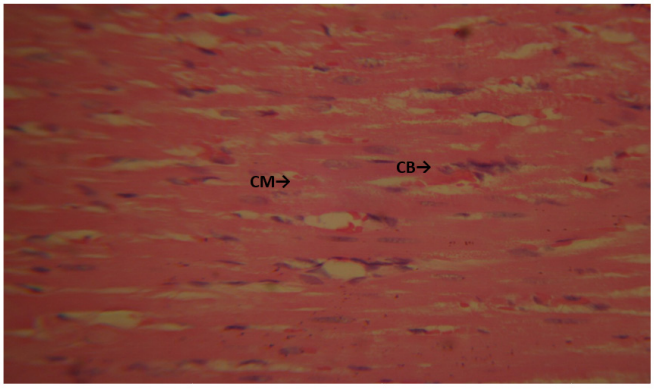


Figure 17: Photomicrograph of section of the standard group treated with glimepiride diabetic heart group showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is no deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation (I); this group shows near-normal heart

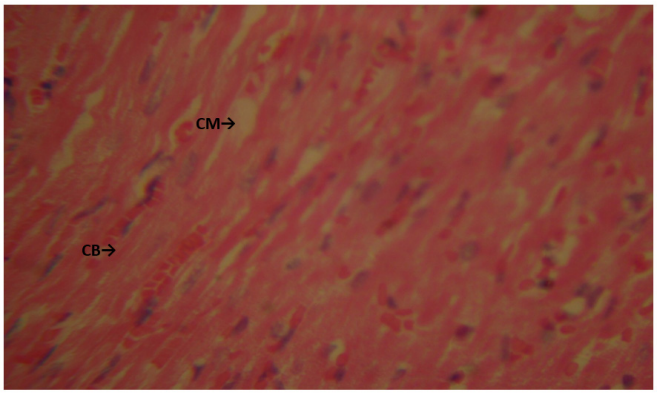


Figure 18: Photomicrograph of section of the diabetic heart treated with ethanol extract of *S. auriculata* leaf (150 mg/kg of body weight) group showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is less degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N); contracture bands (CB), inflammation (I); this group shows near-normal heart

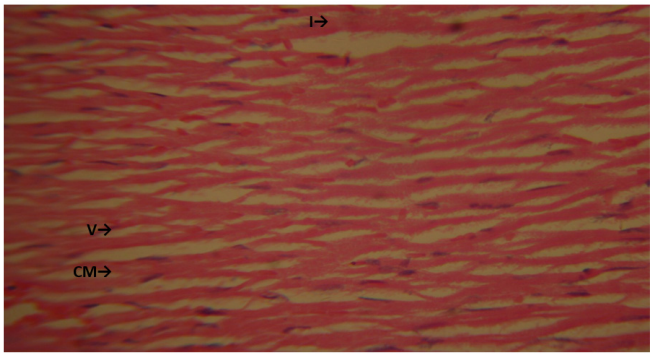


Figure 19: Photomicrograph of section of the diabetic heart treated with ethanol extract of *P. emblica* L. fruit (150 mg/kg of body weight) showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is less degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation (I); this group shows near-normal heart

seeds (150 mg/kg of body weight) and its combination showed significant effectiveness in reducing these parameters in urine.

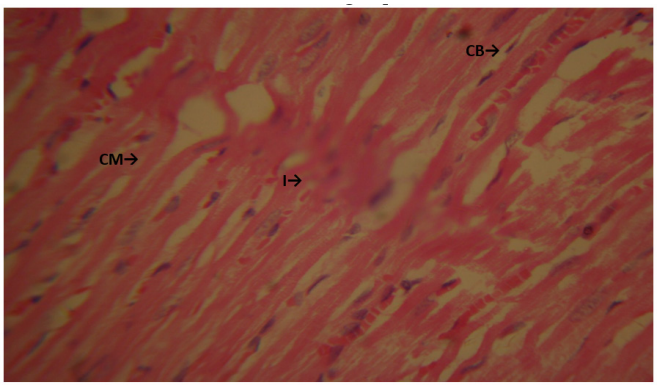


Figure 20: Photomicrograph of section of the diabetic heart treated with ethanol extract of *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is less degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation (I); this group shows near-normal heart

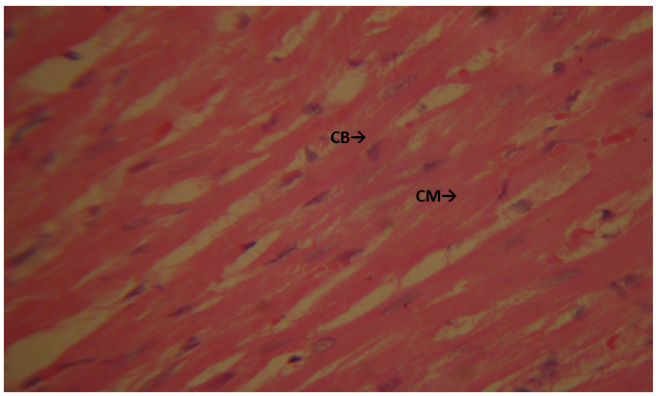


Figure 21: Photomicrograph of section of diabetic heart, treated with combination of ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) showing cardiomyocytes (CM); 150x (after 7 weeks' treatment) there is less degree of deterioration by loss of contractile protein (LCP), vacuolization (V), myelin formations (MF), myocytolysis (MCL), necrosis (N), contracture bands (CB), inflammation(I); this group shows near-normal heart

Diabetic rats treated with ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination showed a reduction in albumin excretion rate, serum creatinine rate, blood urea nitrogen, fasting blood glucose, and renal structural changes. There were also reported marked changes in albuminuria, proteinuria, which is a marker and potential contributor to renal injury accompany diabetic nephropathy. Interventions that have ameliorated the progression of DN have been associated with a reduction in urinary protein excretion. Finally, the significant effect of combined therapy could be a result of synergistic/ potentiative action in diabetic nephropathy and able to target multiple mechanisms involved in the pathophysiology of diabetic nephropathy.

CONCLUSION

In conclusion, the significant effect ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels

seeds (150 mg/kg of body weight) and its combination in diabetic complications like nephropathy and cardiomyopathy in rats was observed, the significant effect could be a result of synergistic/ potentiative action of its combinations, since they contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of diabetic complications like nephropathy and cardiomyopathy. Ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination showed no weight gain. In summary, ethanol extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination treatment reversed the alteration in biochemical parameters. Morphological changes in myocardium and kidney, and improvement of the general behavioral parameters occurs in ethanolic extract of *S. auriculata* leaf, *P. emblica* L. fruit, and *S. cumini* (L.) Skeels seeds (150 mg/kg of body weight) and its combination-treated alloxan-induced diabetic rats. So, the combination of all the three plant parts can be formulated and can be effective in diabetic patients suffering from diabetic complications like nephropathy and cardiomyopathy.

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